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Supplemental Information

Blood-Brain Barrier Breakdown in the Aging Human Hippocampus

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Supplementary Figures

Fig. S1. Regional blood-brain barrier integrity in the living human brain during normal aging (related to Figure 1).

Fig. S2. Correlation between albumin cerebrospinal fluid to plasma quotient (Q_{alb}) and the blood-brain barrier K_{trans} values during normal aging and aging associated with mild cognitive impairment (related to Figure 2).

Fig. S3. Hypoxia and amyloid β-peptide (Aβ) lead to shedding of soluble form of platelet-derived growth factor receptor β (sPDGFRβ) from cultured human pericytes (related to Figure 4).

Fig. S4. Cerebrospinal fluid levels of multiple cell-type specific molecular biomarkers do not show correlation with the blood-brain barrier K_{trans} values in the hippocampus (related to Figures 2 and 4).

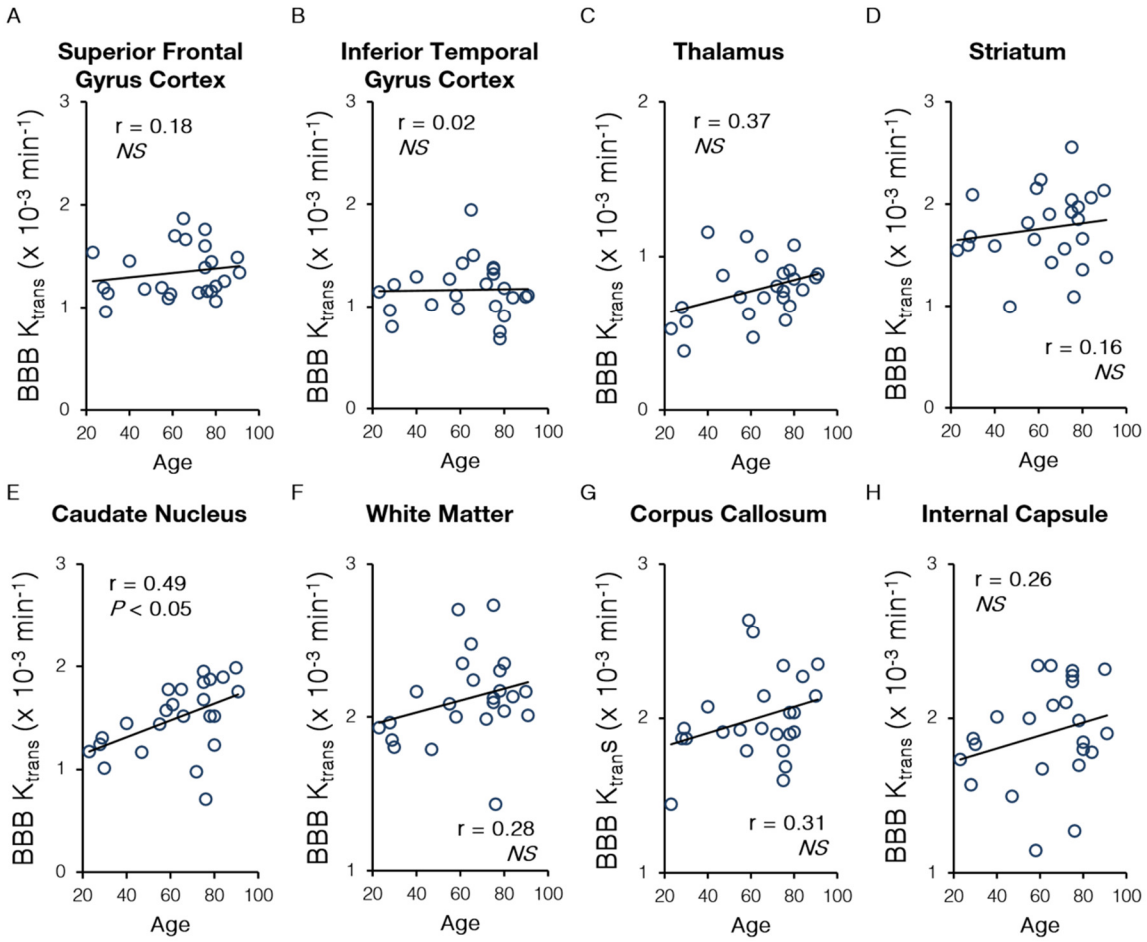

Fig. S1. Regional blood-brain barrier integrity in the living human brain during normal aging (related to Figure 1). (**A-H**) Normal aging did not result in an increase in the BBB permeability K_{trans} constant in multiple gray matter regions (*e.g.*, superior frontal and inferior temporal cortical gyri, thalamus, striatum) and/or in the white matter areas (*e.g.*, subcortical white matter fibers, corpus callosum, internal capsule), except for the hippocampus shown in main Figure 1 and the caudate nucleus (**E**). Single data points for the BBB K_{trans} constant for 24 individuals for different brain regions were plotted against age; r = Pearson's coefficient. *NS*, non-significant; $P < 0.05$ for the caudate nucleus.

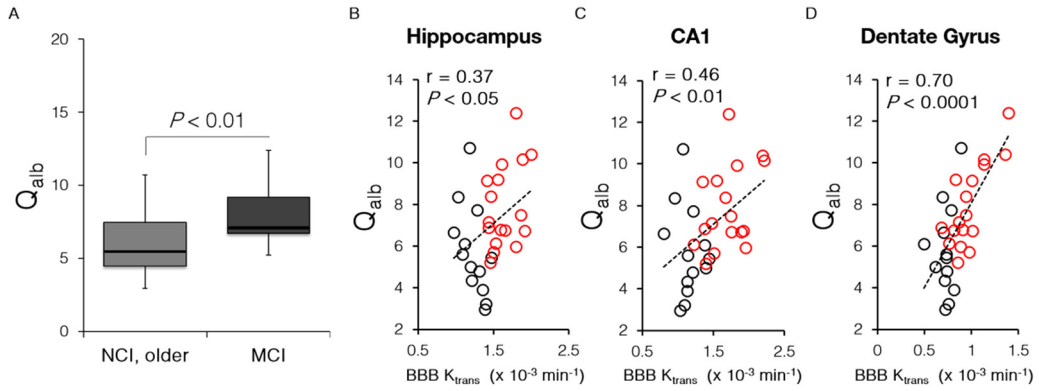

Fig. S2. Correlation between albumin cerebrospinal fluid to plasma quotient (Q_{alb}) and the blood-brain barrier K_{trans} values in the hippocampus and its subfields during normal aging and aging associated with mild cognitive impairment (related to Figure 2). (**A**) An increase in Q_{alb} in individuals with mild cognitive impairment (MCI; n=17) compared to age-matched individuals with no cognitive impairment (NCI, older; n=14). Boxplots represent the median (dark horizontal line), with the box representing the 25th and 75th percentiles, the whiskers the 5th and 95th percentiles. *P*, significance by a Student's *t*-test. (**B-D**) Single data points for Q_{alb} from 31 individuals with no cognitive impairment (n=14, black) or mild cognitive impairment (n=17, red) were plotted against the blood-brain barrier (BBB) K_{trans} constant in the entire hippocampus (**B**), its CA1 region (**C**), and dentate gyrus (**D**); r = Pearson's coefficient; *P*, significance; *NS*, non-significant.

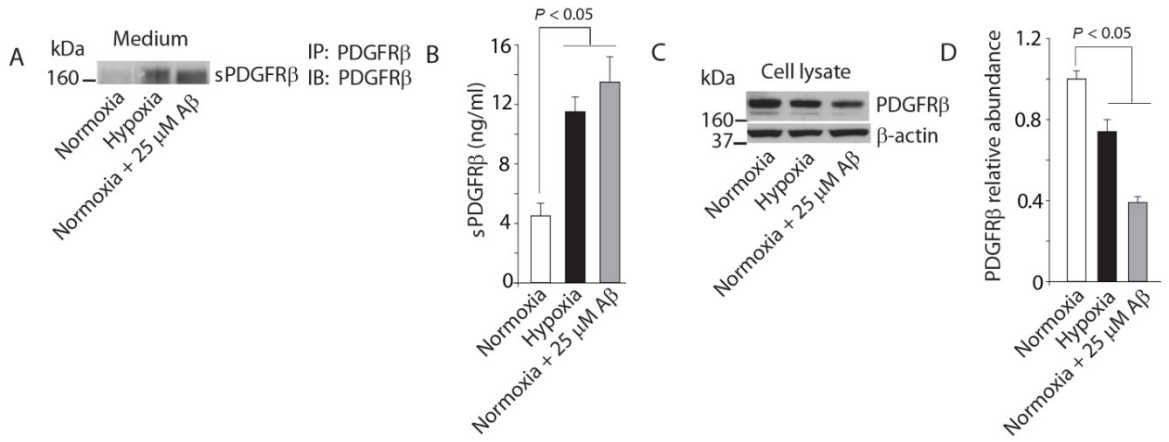

Fig. S3. Hypoxia and amyloid β-peptide (Aβ) lead to shedding of soluble form of platelet-derived growth factor receptor β (sPDGFRβ) from cultured human pericytes (related to Figure 4). Pericytes were treated with 25 μM Aβ40 or exposed to severe hypoxia (1% oxygen) for 48 h. (**A-B**) A shedded form of the PDGFRβ receptor (*i.e.*, sPDGFRβ) was detected in the medium by immunoprecipitation followed by a quantitative Western blotting analysis using a PDGFRβ extracellular domain-specific antibody. The levels of sPDGFRβ in the medium were expressed in ng/ml. (**C-D**) Cell lysates were blotted with a PDGFRβ intracellular domain-specific antibody. The signal intensity from PDGFRβ bands was analyzed by scanning densitometry relative to β-actin. All values are means ± SEM from 3 independent cultures determined in triplicate; *P*, significance by a Student's *t*-test. The relative levels of the cellular PDGFRβ were significantly decreased after hypoxia and Aβ (**C-D**) corresponding to increases in the shedded form of the receptor sPDGFRβ (**A-B**).

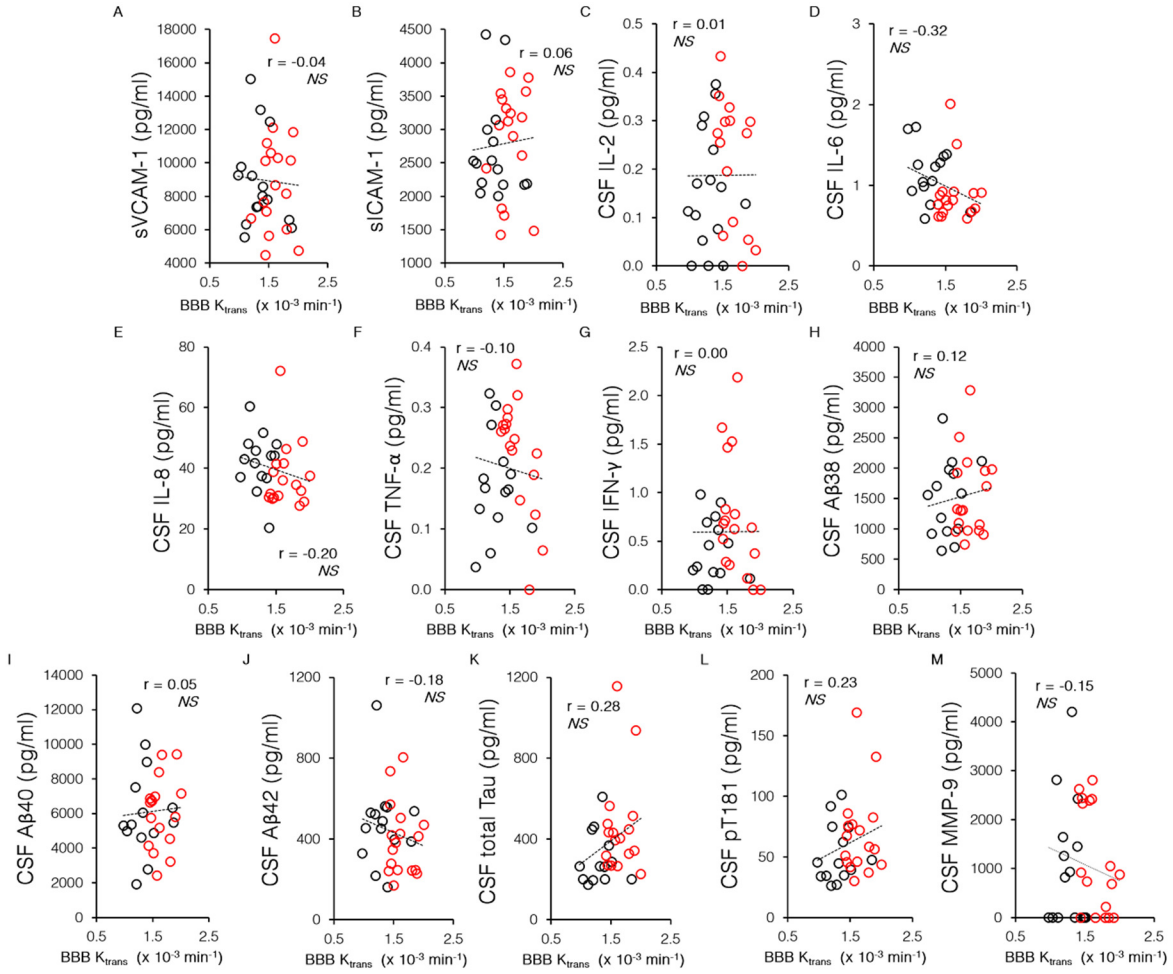

Fig. S4. Cerebrospinal fluid levels of multiple cell-type specific molecular biomarkers do not show correlation with the blood-brain barrier K_{trans} values in the hippocampus (related to Figures 2 and 4). Single data points for CSF biomarkers of *endothelial cell injury* including soluble vascular cell adhesion molecule-1 (sVCAM-1) (**A**), and soluble intercellular cell adhesion molecule-1 (sICAM-1) (**B**), *inflammatory response* (interleukins IL-2, IL-6, IL-8, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) (**C-G**), Aβ levels (Aβ38, Aβ40, and Aβ42) (**H-J**), *neuronal injury* (total Tau, and phosphorylated Tau (pT181) (**K-L**), and the active form of matrix metalloproteinase-9 (MMP-9) (**M**) cerebrospinal fluid levels from 32 individuals including participants with no cognitive impairment (n=15, black) or mild cognitive impairment (n=17, red) were plotted against the blood-brain barrier (BBB) K_{trans} constant in the entire hippocampus; r = Pearson's coefficient; *NS*, non-significant.

Supplementary Table

Supplementary Table 1. Summary table showing the blood-brain barrier K_{trans} values in individuals with no cognitive impairment, mild cognitive impairment and multiple sclerosis (related to Figure 1).

Supplementary Table 1. Summary table showing the blood-brain barrier K_{trans} values in 8 brain regions in individuals with no cognitive impairment (NCI), young (n=6, ages 23-47, both genders) and older (n=18, ages 55-91, both genders), mild cognitive impairment (MCI) (n=20, ages 55-85, both genders) and multiple sclerosis (MS) (n=19, ages 26-53, both genders) (related to Figure 1). ([a], $P < 0.05$ NCI, older compared to NCI, young; [b], $P < 0.001$ MS compared to NCI, young by ANOVA followed by Tukey's post hoc tests). See Table 1.

	Blood-Brain Barrier K_{trans} (x 10^{-3} min^{-1})			
	NCI, young	NCI, older	MCI	MS
Cortex Superior Frontal Gyrus	*1.25 ± 0.21*	*1.38 ± 0.26*	*1.49 ± 0.31*	*1.36 ± 0.21*
Cortex Inferior Temporal Gyrus	*1.08 ± 0.18*	*1.19 ± 0.29*	*1.27 ± 0.25*	*1.14 ± 0.18*
Thalamus	*0.70 ± 0.28*	*0.81 ± 0.17*	*0.89 ± 0.24*	*0.80 ± 0.16*
Caudate Nucleus	*1.22 ± 0.15*	*1.59 ± 0.34* [a]	*1.76 ± 0.31*	*1.28 ± 0.31*
Striatum	*1.58 ± 0.35*	*1.83 ± 0.36*	*1.85 ± 0.18*	*1.62 ± 0.17*
White Matter	*1.92 ± 0.14*	*2.19 ± 0.18*	*2.30 ± 0.36*	*2.53 ± 0.27* [b]
Corpus Callosum	*1.85 ± 0.21*	*2.05 ± 0.29*	*2.19 ± 0.33*	*2.33 ± 0.31* [b]
Internal Capsule	*1.75 ± 0.19*	*1.95 ± 0.35*	*2.02 ± 0.18*	*2.15 ± 0.34* [b]

Values are means ± standard deviation. For the N number please see the caption.

Supplemental Experimental Procedures

Study participants

NCI and MCI participants were recruited through the University of Southern California Alzheimer's Disease Research Center (USC ADRC) and Huntington Medical Research Institute (HMRI), Pasadena. MS patients were recruited from USC Multiple Sclerosis Comprehensive Care and Research Group Clinic. The DCE-MRI procedure was approved by the USC Institutional Review Board (IRB) and HMRI IRB. Lumbar puncture was approved for older NCI and MCI participants by the USC IRB and HMRI IRB.

We enrolled 64 subjects including 24 cognitively normal both genders ages 23-91 including 6 younger NCI (ages 23-47), 18 older NCI (ages 55-90), 21 MCI (ages 55-85) and 19 MS (ages 26-48) (Table 1). MS cases had a diagnosis of the relapsing remitting MS and met McDonald Criteria (Polman et al., 2011). We selected a younger group of MS patients without history of cognitive complaints that were age-matched to younger NCI controls and were on disease modifying treatment.

Neuropsychological Evaluations

Older NCI and MCI participants were evaluated using the Uniform Data Set (UDS) (Morris et al., 2006; Weintraub et al., 2009). In addition, the following neuropsychological tests were given: California Verbal Learning Test, block design, letter-number sequencing, letter fluency, and token test. Cognitively intact NCI participants were defined by CDR=0 and neuropsychological test scores within normal limits; MCI participants were defined by CDR=0.5 and impairment in neuropsychological test scores in one or more cognitive domains.

Exclusion Criteria

We excluded volunteers with ***1***) dementia (CDR≥1), head injury with loss of consciousness > 15 minutes, stroke, or substance abuse, or ***2***) current: organ failure, psychiatric or neurological disorders that might produce dementia symptoms, hydrocephalus, B12 deficiency, hypothyroidism, contraindications to MRI, and medication use likely to affect brain function. MS cases with confirmed cognitive complaints described as cognitive slowing and difficulty with multitasking were excluded from the study.

Procedures

DCE-MRI scans were performed in 64 participants. Lumbar puncture was performed in 32 older NCI and MCI participants. All analyses were performed by an investigator blinded to the experimental conditions.

Magnetic Resonance Imaging

T2-weighted images through the hippocampi and baseline coronal T1-weighted maps acquired using a T1-weighted 3D spoiled gradient echo (SPGR) pulse sequence were obtained on a GE 3T HDXT MR scanner. Gadolinium-based contrast agent (MultiHance®, Bracco, Princeton, New Jersey) (0.05 mmol/kg) was administered intravenously. Coronal DCE-MRI scans were acquired

using a T1-weighted 3D SPGR pulse sequence repeated for 16 minutes with 15.4 seconds temporal resolution per image. Voxel size was 0.625 x 0.625 x 5 mm.

Quantification of the Blood-Brain Barrier Permeability

Post-processing analysis was performed using our in-house scripts running with Matlab. The Patlak linearized regression mathematical analysis (Patlak and Blasberg, 1985; Taheri et al., 2011a, 2011b, 2013) was modified to generate the BBB permeability K_{trans} maps with high spatial and temporal resolutions allowing not only simultaneous measurements of the regional BBB permeability in different WM and grey matter regions, but also accurate calculations of the K_{trans} values in anatomical regions as small as the subdivisions of the hippocampus. In contrast to previous studies using an average value from the superior sagittal venous sinus to determine tracer concentration in blood (Taheri et al., 2011a, 2011b, 2013; Larsson et al., 2009), in the present study we determined in each individual the arterial input function (AIF) from the common carotid artery. In a few cases when the common carotid artery was not clearly visible a nearby large vessel was used. Individual AIF measurements are important particularly if the studied population diverges by age as changes in blood volume and flow may affect AIF and the K_{trans} measurements.

The present analysis requires that the tracer's diffusion across the BBB remains unidirectional during the acquisition time. The total tracer concentration in the tissue, $C_t(t)$, can be described as a function of the capillary concentration $C_p(t)$, the intravascular blood volume v_p, and a transfer constant K_{trans} that represents the flow from the intravascular to the extravascular space using equation (*1*).

$$C_t(t) = K_{trans} \int_0^t C_p(u)\, du \; + \; v_p C_p(t) \qquad (1)$$

A statistically significant intersubject variability in the measurement of v_p was not observed.

Hippocampus Volumetric Analysis

We used SMP8 to segment T2-weighted images.

Molecular Biomarkers in the Cerebrospinal Fluid

Albumin quotient

The CSF/plasma albumin quotient was calculated as Q_{alb}= CSF albumin (mg/L)/plasma albumin (g/L). Enzyme-linked immunosorbent assay (ELISA) was used to determine CSF and plasma albumin (catalog no. E-80AL, Immunology Consultant Laboratories, Portland, OR).

Quantitative Western Blotting of sPDGFRβ

The quantitative Western blot analysis was used to detect sPDGFRβ in human and mouse CSF (ng/mL). Standard curves were generated using recombinant human (Cat. No. 385-PR-100/CF, R&D Systems, Minneapolis, MN) and mouse (Cat. No. 1042-PR-100, R&D Systems) PDGFRβ.

Endothelial markers

Meso Scale Discovery (MSD) assay was used to determine sICAM-1 and sVCAM-1 CSF levels (catalog no. K15198D, MSD, Rockville, MD).

Inflammatory markers

MSD assay (catalog no. K15049G, MSD, Rockville, MD) was used to determine IL-2, 6, 8 and 10, tumor necrosis factor α and interferon γ CSF levels.

Amyloid β-peptide
MSD assay (catalog no. K15199G, MSD, Rockville, MD) was used to determine CSF levels of Aβ38, 40 and 42.

Tau
MSD assay (catalog no. K15121G, MSD, Rockville, MD) was used to determine CSF levels of total tau. Phosphorylated tau (pT181) was determined by ELISA (catalog no. 81581, Innotest, Belgium).

Matrix metalloproteinase-9
Active form of MMP-9 was determined by ELISA (catalog no. 72017, AnaSpec, Fremont, CA).

Human Pericyte Cultures

Human brain microvascular pericytes were isolated and characterized from cortical tissue from patients who underwent brain surgery for epilepsy at the University of Rochester Medical Center as previously described (Zhu et al., 2010). Pericyte cultures were subjected to either hypoxia (1% O_2 for 48 h) (Bell et al., 2009) or Aβ (25 μM Aβ40 for 48 h) (Sagare et al., 2013) as we described. The shedded sPDGFRβ in the medium and PDGFRβ in cell lysates were determined by immunoblotting as described above.

Statistical Analysis

Sample sizes were calculated using nQUERY assuming a two-sided alpha-level of 0.05, 80% power, and homogeneous variances for the 2 samples to be compared. Using the means and common standard deviation for the K_{trans} constant from the pilot data we determined that the sample sizes from 6-9 are sufficient to detect a significant effect $\geq$ 30% between the studied groups. Our current sample sizes for the K_{trans} comparisons varied between 6 and 21, thus satisfying a reliable measurement of the predicted effect as defined above. Using a similar nQUERY analysis and the means and standard deviation for CSF/albumin ratio and other studied CSF biomarkers based on our published (Halliday et al., 2013) and preliminary data the sample sizes to detect the differences between older NCI and MCI groups $\geq$ 30% varied between 5 and 8. Our actual sample sizes for CSF biomarkers were between 15 and 17.

Statistical analysis was performed using Graphpad Prism software (Graphpad prism 5.0; Graphpad Software, La Jolla, CA, USA). Correlations between the BBB K_{trans} values and age (years) or molecular biomarkers (ng or pg/mL) were studied by the Pearson's correlation analysis. F test was conducted to ensure that the data meet the assumptions of the tests. The variance was similar between the groups that were statistically compared. A multifactorial analysis of variance (ANOVA) followed by Tukey's post hoc tests was used to compare BBB K_{trans} values and hippocampal volumes (mm^3). An unpaired Student's *t*-test was used to compare CSF biomarkers between older NCI and MCI groups. Boxplots were used to show the median values (dark horizontal line), with the box representing the 25^{th} and 75^{th} percentiles, and the whiskers the 5^{th} and 95^{th} percentiles. All analyses were performed by an investigator blinded to the experimental conditions. A P value < 0.05 was considered statistically significant.